

CLAIMS

What is claimed is

1. A method for protecting RNA from enzymatic degradation by RNases, the method comprising:
 - (a) to a first solution containing RNA or to which RNA will subsequently be added, adding a second solution, the second solution comprising an amount of an RNase inhibitor protein disposed in a buffer that contains or is devoid of reducing agents, to yield a mixture, wherein the amount of RNase inhibitor protein in the second solution is sufficient to protect RNA from enzymatic degradation by RNases; and then
 - (b) heating the mixture of step (a) to a temperature no less than about 50°C for a time sufficient to inhibit RNase activity present in the mixture; whereby RNA present in the mixture or subsequently added to the mixture is protected from enzymatic degradation by RNases.
2. The method of claim 1, wherein in step (b), the mixture is heated to a temperature no less than about 55°C.
3. The method of claim 1, wherein in step (b), the mixture is heated to a temperature greater than 65°C.
4. The method of claim 1, wherein in step (a), the RNase inhibitor protein is derived from a mammalian source.

5. The method of claim 1, wherein in step (a), the RNase inhibitor protein is derived from porcine, rat, human placental, or recombinant human placental sources.
6. The method of claim 1, wherein in step (b), the mixture does not contain RNA and further wherein the mixture is heated to a temperature no less than about 90°C.
7. The method of claim 1, wherein in step (b), the mixture is heated for at least about twenty (20) seconds.
8. The method of claim 1, wherein in step (b), the mixture is heated for at least about five (5) minutes.
9. The method of Claim 1, which is a method of protecting RNA from enzymatic degradation by RNase A, RNase B, RNase C, and RNase I.
10. A method of inactivating RNases in a first solution containing RNA and suspected of containing RNases, the method comprising:
 - (a) to the first solution, adding a second solution comprising an RNase inhibitor protein deposited in a buffer that contains or is devoid of reducing agents to yield a mixture; and then
 - (b) heating the mixture of step (a) to a temperature of at least about 50°C for a time sufficient to inhibit RNase activity present in the mixture; whereby RNases present in the first solution, if any, are inactivated.
11. The method of claim 10, wherein in step (b), the mixture is heated to a temperature no less than about 55°C.

12. The method of claim 10, wherein in step (b), the mixture is heated to a temperature greater than 65°C.
13. The method of claim 10, wherein in step (a), the RNase inhibitor protein is derived from a mammalian source.
14. The method of claim 10, wherein in step (a), the RNase inhibitor protein is derived from porcine, rat, human placental or recombinant human placental sources.
15. The method of claim 10, wherein in step (b), the mixture is heated for at least about twenty (20) seconds.
16. The method of claim 10, wherein in step (b), the mixture is heated for at least about five (5) minutes.
17. The method of Claim 10, which is a method of inactivating any RNase A, RNase B, RNase C, and RNase I present in the first solution.
18. A method of storing RNA under conditions that protect the RNA from enzymatic degradation by RNases, the method comprising:
 - (a) to a first solution containing isolated RNA or to which isolated RNA will subsequently be added, adding a second solution comprising an RNase inhibitor protein in a buffer that contains or is devoid of reducing agents, to yield a mixture; and then
 - (b) heating the mixture of step (a) to a temperature of at least about 50°C for a time sufficient to inhibit RNase activity present in the mixture; and then
 - (c) cooling the mixture.

19. The method of claim 18, wherein in step (b), the mixture is heated to a temperature no less than about 55°C.
20. The method of claim 18, wherein in step (b), the mixture is heated to a temperature greater than 65°C.
21. The method of claim 18, wherein in step (a), the RNase inhibitor protein is derived from a mammalian source.
22. The method of claim 18, wherein in step (a), the RNase inhibitor protein is derived from porcine, rat, human placental, or recombinant human placental sources.
23. The method of claim 18, wherein in step (b), the mixture does not contain RNA and further wherein the mixture is heated to a temperature no less than about 90°C.
24. The method of claim 18, wherein in step (b), the mixture is heated for at least about twenty (20) seconds.
25. The method of claim 18, wherein in step (b), the mixture is heated for at least about five (5) minutes.
26. A method of performing RT-PCR and quantitative RT-PCR, the method comprising:
 - (a) prior to undergoing thermal cycling, adding to an RT-PCR reaction cocktail containing RNA or to which RNA will subsequently be added, an amount of a solution comprising an RNase inhibitor protein in a buffer that contains or is devoid of reducing agents, to yield a mixture, wherein the amount of the

solution added is sufficient to protect any RNA present in the RT-PCR reaction cocktail from enzymatic degradation during a first round of thermocycling; and then

- (b) adding RNA template to the mixture of step (a) if RNA is absent, and then conducting an RT-PCR reaction on the mixture of step (a), whereby RNA in the mixture is protected from enzymatic degradation by RNases present in the RT-PCR reaction cocktail and is further protected from enzymatic degradation by RNases.

- 27. The method of claim 26, wherein after step (a) and prior to step (b), the mixture is heated to a temperature no less than about 55°C.
- 28. The method of claim 26, wherein after step (a) and prior to step (b), the mixture is heated to a temperature greater than 65°C.
- 29. The method of claim 26, wherein after step (a) and prior to step (b), the mixture is heated to a temperature no less than about 70°C.
- 30. The method of claim 26, wherein in step (a), the RNase inhibitor protein is derived from a mammalian source.
- 31. The method of claim 26, wherein in step (a), the RNase inhibitor protein is derived from porcine, rat, human placental, or recombinant human placental sources.
- 32. The method of claim 26, wherein in step (a) the RT-PCR reaction cocktail does not contain RNA; and after step (a) and prior to step (b), the mixture is heated to at least about 90°C.

33. A method of performing RT-PCR and quantitative RT-PCR, the method comprising:
- (a) to an RT-PCR reagent mixture, adding a first solution containing an RNase inhibitor protein in a buffer, the buffer containing or being devoid of reducing agents, to yield a second solution; and
 - (b) heating the second solution to at least about 55°C for a time sufficient to inhibit RNase activity present in the second solution; and then
 - (c) adding RNA to the second solution to yield an RNA mixture; and then
 - (d) conducting an RT-PCR reaction on the RNA mixture of step (c); whereby the RNA in the RNA mixture is protected from enzymatic degradation by RNases present in the second solution and whereby the RNA in the mixture is further protected from RNases during the RT-PCR reaction.
34. The method of claim 33, wherein in step (b), the second solution is heated to a temperature no less than about 70°C.
35. The method of claim 33, wherein in step (b), the second solution is heated to a temperature no less than about 90°C.
36. The method of claim 33, wherein in step (a), the RNase inhibitor protein is derived from a mammalian source.
37. The method of claim 33, wherein in step (a), the RNase inhibitor protein is derived from porcine, rat, human placental, or recombinant human placental sources.
38. The method of claim 33, wherein in step (b), the mixture is heated for at least about twenty (20) seconds.

39. The method of claim 33, wherein in step (b), the mixture is heated for at least about five (5) minutes.
40. A method of inactivating a prokaryotic or plant RNase comprising:
- (a) to a first solution suspected of containing a prokaryotic or plant RNase, adding a second solution comprising an RNase inhibitor protein in a buffer that contains or is devoid of reducing agents, to yield a mixture; and then
 - (b) heating the mixture of step (a) to a temperature of at least about 55°C for a time sufficient to inhibit prokaryotic or plant RNase activity present in the mixture, whereby prokaryotic and plant RNase present in the first solution is inactivated.
41. The method of claim 40, wherein in step (a), the RNase inhibitor protein is derived from a mammalian source.
42. The method of claim 40, wherein in step (a), the RNase inhibitor protein is derived from porcine, rat, human placental, or recombinant human placental sources.
43. The method of claim 40, wherein in step (b), the mixture is heated for at least about twenty (20) second.
44. The method of claim 40, wherein in step (b), the mixture is heated for at least about five (5) minutes.
45. The method of claim 40, wherein in step (a), the first solution is suspected of containing *E. coli* RNase; and in step (b), the mixture is heated for a time sufficient to inhibit *E. coli* RNase activity present in the mixture.